Improving the Efficiency of Photosynthesis

The opportunity exists to increase crop productivity by regulating wasteful respiratory processes.

Israel Zelitch

"It is the business of agriculture to collect and store solar energy as food energy in plant and animal products." This definition of agriculture comes from a recent report on agricultural production efficiency (*1*), and it seems most appropriate when one considers that 90 to 95 percent of the dry weight of plants is derived from photosynthetically fixed CO_2. Photosynthesis converts electromagnetic energy present in the visible portion of sunlight into the chemical energy needed by green leaves to reduce CO_2 and synthesize our food materials. The practice of agriculture also requires expenditures of cultural energy in the form of labor, fuel, tractors, fertilizers, and pesticides. The amount of cultural energy expended by modern agriculture to obtain food calories varies greatly from crop to crop (*2*). Some forms of cultural energy are already or may soon become in short supply, but sunlight and CO_2, the most important ingredients in the energy conversion in agriculture, are freely available in vast and statistically predictable quantities. They are delivered to field plants without transportation costs of any kind, and we need have no fear of running short of either of them.

Basic research in photosynthesis, especially during the last decade, has revealed a number of possible approaches toward increasing the efficiency of CO_2 assimilation by crops. Efficiency is defined here as the rate of net CO_2 uptake per unit of leaf area or per unit of ground area uncorrected for respiratory losses. Large increases in yields should be obtainable by exploiting this knowledge; it should be possible to diminish wasteful respiratory losses without our having to increase the inputs of cultural energy (*3*). These desirable characteristics can probably be incorporated into plants most easily by utilizing the recent techniques of somatic cell genetics in which tissue cultures are used (*4*), as discussed later herein and by Carlson and Polacco (*4a*).

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Effect of Photochemical Efficiency and Irradiance on Photosynthesis

Photosynthesis takes place in the chloroplasts, and the overall process can be described by the equation:

$$CO_2 + 2H_2O + \text{light} \rightarrow (CH_2O) + H_2O + O_2 + 112 \text{ kcal/g-atom (of C)}$$

The photochemical energy is used in the first stage to remove electrons from water and produce O_2 and a weak reductant. A second photoact is involved in the further transport of electrons to nicotinamide adenine dinucleotide phosphate (NADP); the reducing power, ultimately in the form of NADPH, together with adenosine triphosphate (ATP) produced by photophosphorylation during electron transport, is used to reduce CO_2 to the level of carbohydrate (CH_2O).

Experiments on the effect of irradiance on CO_2 assimilation in leaves of many species show that at low intensities (less than 3 percent of full sunlight) photosynthesis is linear with irradiance (*3*); thus the photochemistry limits the photosynthetic rate at low irradiance. The efficiency of light energy conversion, expressed as the amount of CO_2 absorbed per quantum of energy absorbed, or as calories of carbohydrate produced per calorie of incident visible irradiation, is about 12 percent at these low irradiances. Net CO_2 assimilation is very slow here, and it increases greatly as the light intensity is increased until saturation is reached at 25 percent of full sunlight (except in the most efficient photosynthetic species, such as maize, where saturation is not attained). The efficiency of light energy conversion, however, decreases with increasing irradiance. Thus the maximal daily efficiency of light energy conversion in a field of maize approached only 3 percent, if one neglects respiratory losses (*5*). Photochemical efficiency is therefore not directly related to net CO_2 assimilation rates; on the contrary, the rate of CO_2 assimilation increases greatly with increasing irradiation and diminishing photochemical efficiency. Since there is already a wide difference in the efficiency of CO_2 uptake between species with presumably similar photochemical efficiencies, as discussed below, I conclude that there is ample opportunity for achieving large increases in CO_2 fixation without affecting the photochemical efficiency.

Rates of photosynthetic electron transport may control photosynthesis under some conditions. In experiments with isolated chloroplasts a faster CO_2 fixation was obtained by increasing photosynthetic electron transport with inhibitors of photophosphorylation (*6*), but it is still uncertain whether electron transport is limiting in leaves. Further evidence that neither the enzymatic rates of carboxylation nor the photochemistry limit photosynthesis at high light intensities in leaves is derived from an evaluation of the magnitude of the various diffusive resistances during transport of CO_2 from the atmosphere to the chloroplast (*3*).

Nevertheless, the total irradiation available for photosynthesis and the duration of photosynthesis are undoubtedly important factors determining final plant productivity. Hence productivity can be increased by planting varieties that have rapid rates of leaf area expansion, by the use of closer plant spacings to capture more sunlight, and by breeding for plants whose leaves have more erect angles of elevation to absorb sunlight more effectively.

The Harvest Index

The harvest index is the percentage of the aerial dry weight of a plant that provides useful food material such as grain or seed. The index is therefore a measure of how the products of photosynthesis are partitioned. Its value can vary from as little as 23 percent to as much as 67 percent (Table 1). Primitive agriculturalists probably inadvertently selected for a greater harvest index when they selected larger seed for planting, and in this century in the United States the change from open-pollinated to hybrid varieties of maize also resulted in an increase in the harvest index from 24 to 43 percent (*7*). Even in recent times the harvest index of different crop varieties is found to differ greatly (Table 1). In wheat it ranged from 23 to 46 percent, while the most efficient partitioning of photosynthate into food is in varieties of dry bean, where the range is 53 to 67 percent. Improving the harvest index will continue to be a worthwhile goal of plant breeders and physiologists, especially as the net photosynthetic CO_2 assimilation is improved.

The Relation between Net Photosynthesis and Productivity

Leaves of efficient crop species such as maize, sugarcane, and sorghum have rapid rates of net CO_2 assimilation at high irradiance in normal air at 25° to 35°C (42 to 85 milligrams of CO_2 per square decimeter of leaf area per hour) (*3*). These species also produce malate or aspartate (4-carbon compounds) as the first detectable product when $^{14}CO_2$ is supplied (*8*), hence they have become known as the C_4 species. Leaves of most other crop species, with few exceptions, assimilate CO_2 at rates about one half or less of the C_4 species. The first product from $^{14}CO_2$ found in the less efficient species is 3-phosphoglyceric acid which is synthesized by the operation of the Calvin cycle pathway (*9*). These species are often called C_3 species.

The maximal rate of photosynthesis (CO_2 uptake at saturating CO_2 concentration and high irradiance) is only slightly faster in maize than in tobacco leaves (*3*), indicating that a greatly different photosynthetic capacity is not responsible for the large differences in net photosynthesis in normal air usually observed between C_3 and C_4 plants.

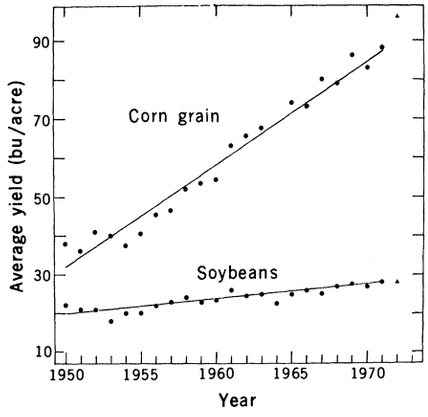

Fig. 1. Average yields per acre of maize grain and soybeans in the United States since 1950 [from (*1*)]. A standard bushel of maize grain weighs 56 pounds, and a standard bushel of soybeans weighs 60 pounds. (One pound of maize grain per acre is almost equivalent to 1 kilogram of grain per hectare.)

Some aspects of the comparative biochemistry of the C_3 and C_4 species as they relate to net CO_2 uptake, glycolic acid synthesis, and especially photorespiration are discussed later in this article.

Since CO_2 fixation provides so much of the dry weight of plants, it is not surprising that rates of net CO_2 fixation by leaves are well correlated with plant productivity. Estimates of the *average* crop growth rate (dry weight produced per square meter of land per week) in the United States for maize silage, sorghum silage, and sugarcane show that these species have two to three times the crop growth rate of photosynthetically less efficient species such as spinach, tobacco, and hay (*3*). Similarly, the literature shows *maximal* growth rates (grams of dry weight produced per square meter of land per day) are about 50 for maize, sugarcane, sorghum, and millet; various other crops with slower rates of net photosynthesis such as rice, sugar beet, and alfalfa have values from 17 to 36 (*3*).

Table 1. The harvest index of some crop species. The harvest index is the percentage of total aerial dry weight at maturity that represents economic yield (grain or seed).

Crop	Harvest index (%)		
	Avg.	Range, different varieties	Reference
Efficient photosynthetic species (C_4)			
Maize, open pollinated, 1920–1926	24		(*7*)
Maize, hybrids, 1958–1959	43		(*7*)
Maize, hybrids	42	38 to 47	(*53*)
Sorghum	41	40 to 41	(*54*)
Other grain species (C_3)			
Rice	51	43 to 57	(*55*)
Barley	48	35 to 52	(*56*)
Oat	41		(*56*)
Wheat	31	23 to 37	(*57*)
Wheat	39	28 to 46	(*56*)
Rye	27	27 to 29	(*56*)
Leguminous species (C_3)			
Dry bean	59	53 to 67	(*58*)
Soybean	32	29 to 36	(*59*)

The average yields of maize grain and soybeans (a C_4 and a C_3 species, respectively) over a period of more than 20 years in the United States are compared in Fig. 1. These are two economically important crops, yet net photosynthesis in maize is at least twice as fast as in soybean leaves at high irradiance. Average maize yields have more than tripled in 20 years since 1950 while soybean yields have increased only about 20 percent. The maize grain yields are now three times greater than soybeans because maize varieties that could utilize nitrogen fertilization were bred; presumably such varieties would maintain high photosynthetic rates during the grain-filling period. Soybeans, on the other hand, do not respond positively to nitrogen fertilization (they fix nitrogen from the air). Even the best technology applied to soybean cultivation does not result in greater yields because the low rate of net photosynthesis in these plants has undoubtedly provided a biological barrier. This barrier in soybean and many other crops is largely explained by the process of photorespiration.

The Characteristics of Photorespiration

Considerable experimental evidence indicates that a large part of the differences in net photosynthesis between the efficient and less efficient species results from the release of photorespiratory CO_2 which occurs rapidly (three to five times faster than "dark" respiration) only in the less efficient species. The losses of carbon by photorespiration are derived from recently fixed photosynthetic compounds, and the process therefore seems wasteful. Hence, the slowing of photorespiration by biochemical or genetic means would be expected to bring about large increases in net photosynthesis and yield.

"Photorespiration" refers to the respiration (usually the CO_2 evolution) in photosynthetic tissues that is specifically

associated with substrates produced during photosynthesis. Photorespiration was first used in its current meaning by Decker and Tió in 1959 (*10*) to account for their observation that leaves of many species exhibited a post-illumination outburst of CO_2, which they correctly attributed to the overshoot of a faster form of respiration that occurred only in the light. At about that time, biochemical evidence was also accumulating that glycolic acid, an early product of photosynthesis (*11*), was rapidly oxidized by a leaf enzyme, glycolate oxidase, and that the rate of glycolic acid synthesis could sustain a rapid respiration in illuminated leaves (*12*). It is now well established that glycolic acid is the primary substrate of photorespiration, and that the characteristics of photorespiration coincide rather precisely with those for the synthesis of glycolic acid and its further oxidation to CO_2.

Photorespiration and glycolic acid metabolism have a number of characteristics in common: (i) an enhanced rate of photorespiration and increased synthesis and oxidation of glycolic acid with increasing O_2 concentrations in the atmosphere; (ii) a similar dependence on light intensity and a requirement for actively photosynthesizing tissues; (iii) a relative independence on CO_2 concentration between zero and 300 parts per million (ppm) and inhibition when the CO_2 concentration is increased above about 2000 ppm; and (iv) a strong temperature dependence. More direct evidence of the involvement of glycolic acid comes from measurement of $^{14}CO_2$ output from ^{14}C-labeled glycolate, and the use of inhibitors of glycolate synthesis and oxidation, and from kinetic experiments with $^{14}CO_2$.

The Measurement and Magnitude of Photorespiration

All assays of photorespiration underestimate its magnitude because the measurements have to be made in the ambient atmosphere surrounding the illuminated leaf, and the main flux of CO_2 during assays is into the leaf. The main methods of detecting and assaying photorespiration, and their limitations, have been discussed elsewhere (*3, 13*). Such assays include the following measurements: the uptake of $^{18}O_2$; the extent of the inhibition of net CO_2 uptake upon raising the O_2 concentration in the atmosphere; the postillumination CO_2 outburst; extrapolation of the curve of CO_2 concentration against net photosynthesis to "zero" CO_2; dilution of the $^{14}CO_2$ specific radioactivity in the ambient atmosphere in a closed system; discrimination against $^{13}CO_2$ as opposed to $^{12}CO_2$ during photosynthesis; the rate of CO_2 released in CO_2-free air (with or without ^{14}C-labeled tissues); the CO_2 compensation point; and the short time of uptake (approximately 15 seconds) of $^{14}CO_2$ minus $^{12}CO_2$ when both are supplied together.

Various assays show that photorespiration in the less efficient photosynthetic species occurs at rates at least 50 percent of net CO_2 uptake (Table 2), while it is barely detectable in the efficient species such as maize. Blocking photorespiration with biochemical inhibitors of glycolic acid synthesis (*14*) or oxidation (*15*) also increases net photosynthesis by 50 percent or more in species with rapid photorespiration but not in maize. Thus the magnitude of photorespiration in C_3 species must be greater than 10 mg of CO_2 per square decimeter per hour; 76 micromoles of CO_2 per milligram of chlorophyll per hour; or 114 mg of CO_2 per gram of fresh weight per hour.

Any proposed biochemical mechanism of photorespiration in a C_3 species must at least be able to account for such high rates of CO_2 production by photorespiration. Several investigators (*16, 17*) have expressed the view that the CO_2 is released in photorespiration by a biochemical mechanism in which the synthesis of glycolic acid occurs exclusively by the ribulose diphosphate oxygenase reaction. Such a hypothesis permits the magnitude of photorespired CO_2 released by the conventional glycolate pathway to be no greater than 14 to 20 percent of the net CO_2 fixed (*16, 17*). Table 2, however, illustrates that by a number of different assays, in different species, and in many laboratories at least 50 percent of the CO_2 assimilated is released and measured during photorespiration, and this is inconsistent with the biochemical scheme presented (*16*). Glycolic acid is synthesized sufficiently rapidly in C_3 species, 70 to 80 μmole per gram of fresh weight per hour (*18*), to account for the results in Table 2.

Table 2. Minimal rate of photorespiration in soybean, sunflower, sugar beet, tobacco, and maize (*13*). The values are minimal and are underestimates because photorespiration is assayed under conditions of high light intensity where the main flux of the gas (CO_2 or O_2) is in the opposite direction.

Method of assay	Temperature (°C)	Net photosynthesis in normal air (mg CO_2/dm²/hour)	Photorespiration (% of net photosynthesis)	Reference
Soybean				
CO_2 release, CO_2-free air*	26	35	46	(*60*)
Postillumination CO_2 burst	25	11	75	(*61*)
CO_2 release, CO_2-free air	30	18	42	(*62*)
Sunflower				
Short-time uptake, $^{14}CO_2$ minus $^{12}CO_2$	25	25	60	(*63*)
$^{14}CO_2$ release, CO_2-free air	25	28	27	(*64*)
Sugar beet				
CO_2 release, CO_2-free air	25	25	47	(*65*)
CO_2 release, CO_2-free air	25	26	40	(*62*)
Tobacco				
CO_2 release, CO_2-free air	25	11	55	(*66*)
Extrapolation of net photosynthesis to "zero" CO_2	25	14	25	(*67*)
Postillumination CO_2 burst	26	17	45	(*68*)
Postillumination CO_2 burst	34	15	66	(*68*)
Maize				
CO_2 release; air passed through leaf	30			(*69*)
CO_2 release, CO_2-free air	35	50		(*62*)
$^{18}O_2$ uptake	31	12	6	(*70*)

* Results are the mean values of 20 varieties recalculated by the authors considering the effects of internal diffusive resistances.

The Synthesis of Glycolic Acid

The rate of glycolic acid synthesis, which occurs largely in leaf chloroplasts, is probably the most important factor controlling photorespiration, but not all of the biochemical reactions responsible for the synthesis of the glycolic acid in any given photosynthetic tissue have been worked out. A num-

ber of reactions are known that produce glycolic acid (Fig. 2), and at least several of these may occur simultaneously.

The important observation was recently made (*19, 20*) that ribulose-1,5-diphosphate carboxylase, the enzyme primarily responsible for CO_2 uptake during photosynthesis, also catalyzes the reaction between the substrate and O_2 to produce phosphoglycolic and phosphoglyceric acids (Fig. 2, reaction a). The properties of the oxygenase reaction have much in common with the well-known inhibition of photosynthetic CO_2 uptake by O_2 (the Warburg effect) (*16, 17*). Leaves contain an active phosphoglycolate phosphatase in their chloroplasts (*21*) that could rapidly produce glycolic acid. Such observations have led some investigators to conclude prematurely that the oxygenase reaction can account entirely for the synthesis of glycolic acid and photorespiration.

There are some difficulties in accepting the ribulose-1,5-diphosphate oxygenase reaction as the major pathway of glycolic acid synthesis in photosynthetic tissue. First, the rates of synthesis by this reaction with isolated systems are generally far too slow to accommodate the minimal photorespiratory rates shown in Table 2. Second, in intact leaves in light there was a rapid incorporation of $^{18}O_2$ into the carboxyl groups of glycine and serine (products of glycolic acid metabolism) as expected (*22*). But even in an atmosphere of 100 percent O_2, which would greatly favor the oxygenase, the specific activity of incorporated $^{18}O_2$ was no greater than one third of that supplied, indicating that approximately one third of the glycolate was produced by the oxygenase reaction in 100 percent O_2 and two thirds by some other reaction. Finally, the product of the oxygenase reaction, phosphoglycolic acid, has not yet been shown to function as an important intermediate in vivo in kinetic experiments with $^{14}CO_2$ in either intact tissues or with isolated chloroplasts. From kinetic experiments in which $^{14}CO_2$ was used with *Chlorella* in 100 percent O_2, it was concluded that about one third to one half of the glycolic acid produced could have arisen from phosphoglycolic acid (*23*). Presumably, an even smaller proportion of the glycolic acid would have come from phosphoglycolic acid in normal air than in 100 percent O_2.

Glycolic acid was synthesized in a reconstructed chloroplast system in the light in the presence of a transketolase substrate, such as ribulose 5-phosphate or fructose 6-phosphate, together with transketolase, NADP, and ferredoxin (*24*) (Fig. 2, reaction b). An oxidant, presumably hydrogen peroxide, from the oxidation of reduced ferredoxin or NADPH was generated during illumination and produced glycolic acid from "active glycolaldehyde" at maximal rates about 10 percent of that needed for photorespiration. Additional support for the direct functioning of intermediates of the Calvin cycle in glycolic acid biosynthesis comes from the demonstration that two carbon fragments from added ribose 5-phosphate and fructose 1,6-diphosphate are directly incorporated into glycolic acid in isolated spinach chloroplasts (*25*).

Active glyoxylate reductase enzymes are known to occur in leaves (*3*) (Fig. 2, reaction c), and glyoxylate may be available for glycolate formation from several pathways including the isocitrate lyase reaction (*26*). The compound [2-^{14}C]glyoxylate is easily converted to [2-^{14}C]glycolate by leaves, and organic acids such as [3-^{14}C]pyruvate are incorporated into [2-^{14}C]glycolate in leaf tissues (*18*). Such observations can be explained by the functioning of the glyoxylate reductase reaction.

A direct but still undefined carboxylation reaction may also produce glycolic acid (Fig. 2, reaction d). When $^{14}CO_2$ and an inhibitor of glycolic acid oxidation were supplied together to illuminated leaves, the accumulated glycolic acid carbon atoms had a specific radioactivity that was about 50 percent of that of the $^{14}CO_2$ supplied and was greater than that in the carboxyl-carbon atom of phosphoglyceric acid (*27*), the first product of photosynthesis. This shows that glycolic acid is synthesized rather directly from fixed CO_2. More recently, in studies with $^{14}CO_2$ fixation in illuminated chloroplasts in the absence of any inhibitors, Robinson and Gibbs (*25*) found that the specific radioactivity of the carbon atoms of [^{14}C]glycolate was between 53 and 71 percent of the $^{14}CO_2$ supplied. These experiments also indicate that a rather direct synthesis of glycolic acid from CO_2 is possible.

Multiple pathways, including the reactions above, probably occur in the same tissue. For example, organic acids such as [2-^{14}C]acetate and [3-^{14}C]pyruvate were incorporated into [2-^{14}C]glycolate with a higher specific radioactivity in maize than tobacco leaves, while $^{14}CO_2$ incorporation into glycolate was much better in tobacco than in maize (*18*). The addition of phosphoenolpyru-

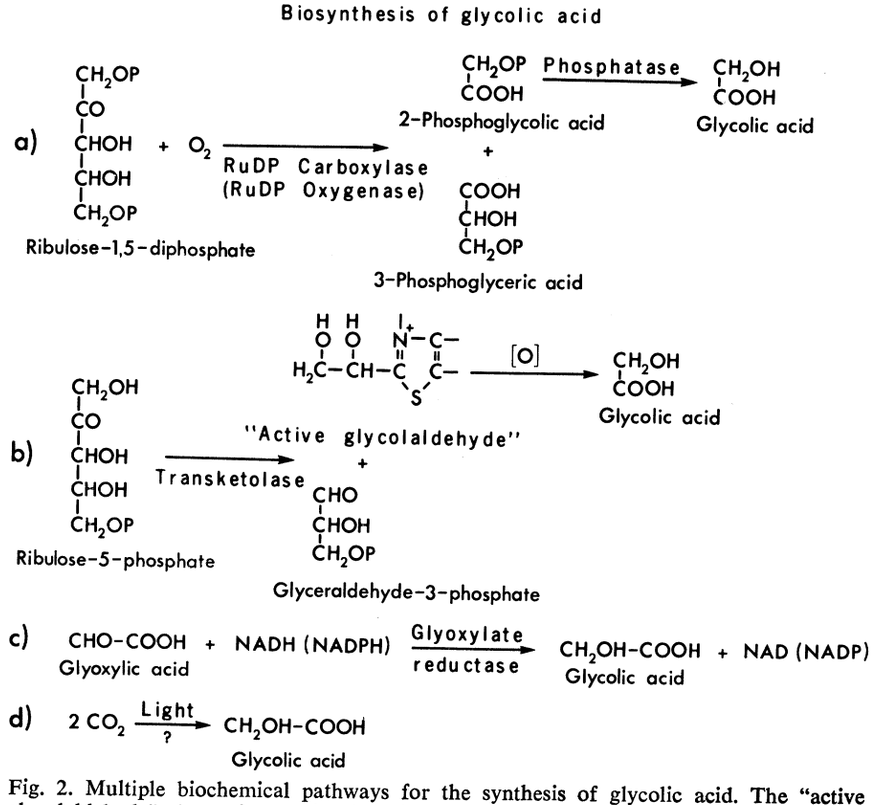

Fig. 2. Multiple biochemical pathways for the synthesis of glycolic acid. The "active glycolaldehyde" shown in reaction b is 2-(α,β-dihydroxyethyl thiamine pyrophosphate).

vate stimulated glycolate synthesis in maize but not in tobacco. Eickenbusch and Beck (*28*) demonstrated the existence of at least two kinds of reactions concerned with glycolic acid synthesis in isolated spinach chloroplasts. The rate of one pathway was unchanged by O_2 concentrations up to that in normal air, while the rate of the second pathway increased linearly when the O_2 concentration was raised above that in the air. Based on changes in the specific radioactivity of various intermediates in maize and sunflower leaves, Mahon *et al.* (*29*) also concluded that there are sources of carbon for glycolate synthesis besides the intermediates of the Calvin cycle.

Glycolic Acid Synthesis and Photorespiration within a Species

When illuminated leaf tissue of a species with rapid photorespiration is placed in a solution of α-hydroxysulfonate, glycolic acid oxidation is blocked and the glycolic acid accumulates at an initial rate (*18*) sufficient to account for the minimal rates of photorespiration shown in Table 2. Under the same conditions, however, maize leaf tissue synthesizes glycolic acid at about one tenth the rate in C_3 species (*14, 18*).

A possible explanation for the slow synthesis in C_4 species is offered in the next section, but it appears that much of the difference in net photosynthesis between C_3 and C_4 species can be explained by the slow rate of glycolic acid synthesis, and hence slower photorespiration, in the efficient photosynthetic species. Placing plants with rapid photorespiration in atmospheres containing less than 2 percent O_2 or in elevated concentrations of CO_2 (at least 0.2 percent) decreases glycolic acid synthesis, decreases photorespiration, and increases the dry weight yield to a level similar to maize (*3*). It therefore would seem most urgent to learn how to diminish the rate of glycolate production by biochemical or genetic means in normal environments in tissues with rapid rates of photorespiration and thus mimic what occurs normally in maize.

Any environmental condition or biochemical inhibitor that interferes with photosynthesis will of course also inhibit glycolic acid synthesis. A specific biochemical inhibitor of glycolic acid synthesis should also be expected to increase net CO_2 assimilation rather than inhibit it. Goldsworthy (*30*) showed that isonicotinic acid hydrazide inhibits photorespiration in tobacco leaves. This occurs because the inhibitor blocks glycolic acid synthesis (*18*), but the effect of this inhibitor on net photosynthesis has not been examined.

Recently I found that glycidic acid, 2,3-epoxypropionic acid, an epoxide similar in structure to glycolic acid, blocks glycolic acid synthesis and not glycolic acid oxidation in tobacco leaf tissue (*14*). Under conditions where glycolic acid synthesis and photorespiration were slowed by about 50 percent by the inhibitor, net photosynthetic CO_2 fixation was increased similarly, by about 50 percent. The inhibitor also blocked glycolic acid synthesis in maize leaf, but had no effect on CO_2 uptake in this tissue, presumably because maize already synthesizes so little glycolic acid. There was no effect of glycidic acid on the isolated ribulose diphosphate carboxylase (oxygenase) reaction, but the biochemical mechanism for inhibition by glycidic acid has not yet been established. The use of this inhibitor has therefore confirmed in an independent manner that slowing glycolic acid synthesis can result in large increases in net photosynthesis in an inefficient photosynthetic species.

Zelitch and Day (*31*) observed that a yellow-leaved variety of tobacco (JWB mutant) had slower rates of net photosynthesis in normal air and a faster photorespiration than its green-leaved siblings (JWB wild). Since JWB mutant plants were altered by a simple genetic change (albeit with pleiotropic effects in this case), this example demonstrated that genetic control was capable of regulating photorespiration within a species. As predicted by our hypothesis, the variety with a slower rate of photorespiration grew more rapidly in a greenhouse environment.

Wilson (*32*) has observed variations in photorespiration within populations of the grass species *Lolium*. We have described the results of pedigree selections on siblings of several generations of normal-appearing tobacco plants (Havana Seed) with slower than usual photorespiration and faster net photosynthesis than is commonly observed for this species (*33*). Superior plants, on selfing, produced about 25 percent of their progeny with slow photorespiration and fast net CO_2 uptake, but the percentage was not increased in several successive generations. It was clearly established, however, that some plants growing side by side showed about 50 percent of the normal rate of photorespiration, similar rates of dark respiration, and an increased net photosynthesis of about 40 percent. Thus, differences in photorespiration undoubtedly can occur within a species, and innovative genetic methods may be required to fix this characteristic in an entire population.

Natural Regulation of Glycolic Acid Synthesis in C_4 Species

Many investigations support the view that, in the leaves of C_4 species, the first carboxylation reaction occurs in the mesophyll cells and that malate or aspartate is then transported to the specialized bundle sheath cells that surround the vascular tissue in these species (*8, 34*). The 4-carbon compound is decarboxylated, and the released CO_2 is fixed once more by reactions associated with the Calvin cycle in the bundle sheath cells. There are still a number of uncertainties and controversies about the C_4 pathway (*13*), but one of its main features involves an increase in CO_2 concentration in the bundle sheath cells.

Hatch (*35*) calculated the sizes of the pools of CO_2 and 4-carbon compounds in these two types of cells in maize leaf from short-term labeling experiments using $^{14}CO_2$ followed by $^{12}CO_2$ on the assumption that the CO_2 pool was restricted to the mesophyll or the bundle sheath compartment. In this way he estimated that the CO_2 concentration in bundle sheath cells was five times greater than in the other photosynthetic cells. High concentrations of CO_2 are known to inhibit glycolic acid synthesis (*3*), and the site of glycolic acid formation in C_4 species is believed to occur only in the bundle sheath cells (*34*). Thus species such as maize probably synthesize glycolate slowly because the anatomical and biochemical compartmentation of their specialized photosynthetic cells cooperate to create an inhibitory environment.

The C_4 species seem to have evolved an unnecessarily complicated and indirect biochemical mechanism for inhibiting glycolic acid biosynthesis and slowing photorespiration. Björkman (*36*) was unable to convert a C_3 species into a C_4 species by producing interspecific hybrids in *Atriplex* using parental plants of one type and the other, and the hybrids of this weed had a decreased net photosynthesis. Neither the superior

C_4 anatomy nor a rapid phosphoenolpyruvate carboxylase activity was sufficient to assure the rapid CO_2 uptake characteristic of C_4 plants. Perhaps this is not surprising since a conversion of a C_3 plant into a C_4 would involve large changes in leaf morphology, chloroplast type, and enzyme activities.

It would seem easier to select for a slower photorespiration in the less efficient photosynthetic species by slowing glycolic acid synthesis more directly, and in this way one should expect to obtain large increases in net photosynthesis without invoking the C_4 system. Indeed, examples of decreased rates of photorespiration within a species have been found, as discussed before, and these superior photosynthetic plants do not utilize the C_4 pathway.

The Oxidation of Glycolic Acid and Its Relation to Photorespiration

In order to be further metabolized, glycolic acid must first be oxidized to glyoxylic acid (the apparent reverse of Fig. 2, reaction c), since no other biochemical reaction is known whereby glycolic acid itself reacts. In higher plants, glycolate oxidase is a flavoprotein that couples with O_2, and the reaction rate is very dependent on the O_2 concentration. This enzyme is located mainly in the peroxisomes of green leaves (*37*).

α-Hydroxysulfonates are aldehyde-bisulfite addition compounds and effective competitive inhibitors of glycolate oxidase (*38*). When a suitable sulfonate is supplied to illuminated leaf tissues, glycolic acid accumulates at initial rates sufficient to account for photorespiration in tobacco and sunflower (*18*). Inhibition of glycolate oxidation in tobacco leaf under suitable conditions in the laboratory also blocks photorespiration and brings about large increases in photosynthetic CO_2 uptake, but the sulfonate does not increase photosynthesis in maize leaf (*15*).

A biochemical sequence known as the glycolate pathway of carbohydrate synthesis exists in leaves (*3, 39*), and this pathway provides the photorespired CO_2. As usually depicted, four molecules of glycolic acid (glyoxylic acid) yield one of glucose and two of CO_2; the CO_2 is believed to arise during the step involving the condensation of two glycine molecules to yield serine. The stoichiometry indicated above permits the loss of only 25 percent of the glycolic acid metabolized as CO_2 and, as already shown in Table 2, photorespiratory CO_2 often accounts for at least 50 percent of net CO_2 fixation during photosynthesis. Thus CO_2 cannot be produced during photorespiration from only the decarboxylation of glycine (*3, 13*). The oxidative decarboxylation of glyoxylic acid by hydrogen peroxide yields formic acid and CO_2, and this reaction can be rapid in illuminated chloroplasts (*40*), can also occur in peroxisomes (*41*), and could account for most of the photorespiratory CO_2. The further oxidation of formic acid can also contribute to the CO_2 produced during photorespiration (*15*).

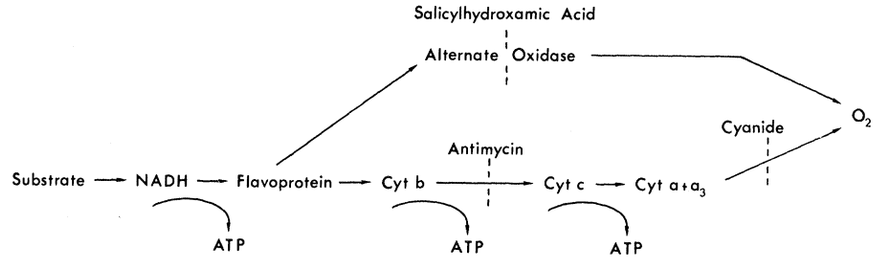

Fig. 3. The normal (antimycin- and cyanide-sensitive) and alternate (salicylhydroxamic acid–sensitive) pathways of electron flow and ATP formation in plant mitochondria (*43*). Abbreviations: NADH, reduced nicotinamide adenine dinucleotide; Cyt, cytochrome.

Leaves of all species appear to have activities of glycolate oxidase in excess of that needed for photorespiration (*3*), and an excess of the other enzymes associated with the glycolate pathway as well (*37*). Since glycolic acid must first be oxidized to be further metabolized, controlling the glycolate oxidase activity by genetic means would not seem to be a reasonable means of controlling photorespiration because glycolate would continue to accumulate. If the utilization of an intermediate in the pathway, such as glyoxylate or glycine, were enhanced, less substrate might become available for decarboxylation during photorespiration. Alternatively, it is conceivable that the concentration of some metabolite in the glycolate pathway, even glycolic acid itself, might exercise a feedback inhibition on glycolic acid synthesis, and this property could be utilized to control photorespiration.

Dark Respiration and Plant Productivity

The respiration of higher plants that is easily measured in darkness takes place primarily, but not exclusively, in the mitochondria. Its biochemistry is similar in plants, animals, and microorganisms. The process provides ATP, largely from the reactions of oxidative phosphorylation, while substrates are oxidized to CO_2. In microorganisms, ATP production often limits growth, and it may be assumed that this is also often true in higher plants. Respiration also provides carbon compounds that are utilized in many biosynthetic pathways that occur in plant cells.

The rate of respiration in photosynthetic tissues in darkness is usually 5 to 10 percent of the CO_2 assimilation in bright light. The lower leaves in a crop are shaded to various degrees and may carry out little photosynthesis, and respiration also occurs in stems, roots, and fruits that usually fix little or no CO_2. There is excellent evidence (*3*) that the reactions of "dark" respiration occur just as rapidly in the light as they do in photosynthetic tissues in darkness, and values in the literature show that 29 to 71 percent of the gross CO_2 fixed during photosynthesis may be lost by the process of dark respiration (*3*).

If part of the dark respiration were wasteful, eliminating it would also bring about large increases in plant productivity. Therefore, it is important to establish how much of the dark respiration is coupled to useful synthetic and growth processes, and what portion might be eliminated. Eliminating wasteful dark respiration might also increase the productivity in the efficient photosynthetic species such as maize.

One well-documented example of a wasteful dark respiration in maize has been described by Heichel (*42*), who compared the net CO_2 exchange and dry weight increase of two inbred varieties grown in the same environment. One variety showed about 50 percent faster gain in dry weight although the rates of net CO_2 assimilation were similar. The faster growing variety lost 26 percent of its gross CO_2 uptake

Table 3. Photosynthetic activity of plant cultures. L/D values are ratios of the rate of $^{14}CO_2$ uptake in the light to the rate of uptake in the dark.

Species	Carbon source for growth	Growth illumination (lux)	Chlorophyll content (μg/g fresh weight)	Demonstration of photosynthesis	Reference
Carrot	1 percent CO_2 in air	10,000	50	40 μmole CO_2 per milligram of chlorophyll per hour	(*47*)
Ruta graveolens	1 percent CO_2 in 99 percent N_2	2,000		L/D = 6.0	(*71*)
Atropa belladonna	Sucrose	6,000	57	L/D = 13.0	(*49*)
Froelichia gracilis	Sucrose	7,000		113 μmole CO_2 per gram fresh weight per hour	(*72*)
Tobacco	2 percent CO_2 in air	900–1,700	61	500 μmole O_2 per milligram of chlorophyll per hour	(*48*)
Tobacco	Sucrose	5,400	27	L/D = 7.0	(*50*)
Tobacco	Sucrose	1,500	13	Delayed chlorophyll fluorescence	(*51*)
Tobacco	0.03 percent CO_2 in air	4,000		L/D = 9.0	(*73*)
	Sucrose	4,000		L/D = 2.0	
Tobacco	0.03 percent CO_2 in air	4,000	25	120 μmole CO_2 per milligram of chlorophyll per hour	(*73*)
	Sucrose	4,000	15	85 μmole CO_2 per milligram of chlorophyll per hour	

during respiration in the dark as compared with 33 percent in the poorer variety. In this example, an appreciable portion of the dark respiration was clearly wasted in the slower growing inbred.

Several possible biochemical explanations could account for a potentially wasteful dark respiration. Some of the respiration may occur by oxidases outside the mitochondria which may not produce ATP, or the oxidations within the mitochondria might not be tightly coupled to ATP synthesis. Finally, it seems likely that some portion of mitochondrial respiration occurs by the recently discovered alternate pathway of electron transport in mitochondria, which produces only one third as much ATP for each pair of hydrogen atoms oxidized as does the conventional pathway (see Fig. 3). This alternate pathway is under genetic control in fungi (*43*) (it is the main pathway in "poky" or slow growing *Neurospora* mutants) and it occurs in higher plants (*44*).

The alternate pathway of respiration is insensitive to antimycin and cyanide, and is specifically inhibited by salicylhydroxamic acid, which does not affect the conventional electron transport system. In isolated mitochondria from a number of plant species and tissues, under conditions of rapid respiration and phosphorylation, the alternate pathway contributed from 1.0 to 100 percent of the respiration, with a value of 15 to 20 percent being most often observed (*44*). Mitochondria from leaves were not examined in this investigation, but it is well known that 50 percent or more of the dark respiration in leaves is cyanide-insensitive (*45*) and presumably occurs by the alternate pathway. Thus, eliminating the alternate pathway, by producing mutants in plant tissue cultures, might be expected to result in greater plant productivity.

The Use of Tissue Cultures to Obtain Desirable Mutants

Recent advances in somatic cell genetics in higher plants (*4*) (see the article by Carlson and Polacco (*4a*) suggest the usefulness of screening large populations of haploid plant cells so that only the desired phenotype (low rates of photorespiration or more efficient dark respiration, for example) will survive. Intact normal plants in which both gene copies will be identical can already be obtained by this technique in many species. The main problem at present is learning how to screen for the desired mutants possessing increased net photosynthesis or productivity, and this approach is now being actively pursued here at our station and undoubtedly elsewhere.

One obvious approach would be to devise selection methods for superior CO_2 uptake in illuminated plant tissue cultures grown on CO_2 as the carbon source, with the hope that such a phenotype would be expressed in the intact plant. Most experienced investigators seem to agree with the statement expressed by Gautheret (*46*) that tissue cultures will not grow on CO_2 alone as a carbon source. There are more recent indications, however, that some cultures can grow on CO_2 and that even cultures grown on sucrose in the light also assimilate some CO_2 photosynthetically (Table 3). Whether cultures can be maintained indefinitely under autotrophic conditions is still not clear. With carrot tissue (*47*) the cells did not survive more than 2 weeks on CO_2, but Chandler *et al.* (*48*) apparently grew tobacco cultures for a considerable time on CO_2 without them differentiating. Light is essential for chloroplast formation and chlorophyll synthesis in tissue cultures (*47*, *49–51*), and Koth (*52*) recently showed that ribulose diphosphate carboxylase activity was present in tobacco cultures after transfer of the tissue to light and that the increase in enzyme activity accompanies the increase in chlorophyll.

An alternative approach would be to select for decreased photorespiration in haploid tissue cultures grown on sucrose by obtaining mutants with decreased rates of glycolic acid synthesis or possessing a more efficient utilization or regulation of intermediates of the glycolate pathway of carbohydrate synthesis. Attempts to achieve this are being made in our laboratory, and Carlson and Polacco (*4a*) describe some experiments with plant tissue cultures aimed at eliminating the inefficient alternate pathway of dark respiration described earlier.

This use of tissue cultures and the application of our knowledge of the biochemistry of carbon compounds related to photosynthesis would seem to offer the greatest promise of enabling us to find methods for increasing productivity in a number of crops. The potential importance of regulating wasteful respiratory processes, as discussed here, represents only one of a number of biochemical strategies that might be applied to the problems of world food production in the future.

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Nitrogen Fixation Research: A Key to World Food?

Investigators in a variety of disciplines are searching for new technologies for producing fixed nitrogen.

R. W. F. Hardy and U. D. Havelka

Population growth and changes in dietary habits accompanying economic growth will more than double the demand for agronomic crops during this quarter century. Among the many factors that could contribute to improving crop yields, increasing the availability of fixed nitrogen to crops is probably of the greatest importance. In the absence of alternative technologies for producing fixed nitrogen, about 200 × 10^6 metric tons of fertilizer nitrogen will be required annually by the end of this quarter century. In 1974, 40 × 10^6 tons of fertilizer nitrogen with an approximate value of $8 billion were used, as opposed to the 3.5 × 10^6 tons that were used annually 25 years ago.

The recent scarcity of nitrogen fertilizers, the high energy requirement for their manufacture, and, most significantly, their increased selling price have produced a tremendous interest in the search for alternative technologies. This interest has permeated even the popular literature, as documented by the following quotation from *Harper's Magazine*, by Horace Freeland Judson (*1*):

. . . a biologist working in Brazil, said she has found several kinds of tropical grasses that grow in symbiosis with N_2-fixing bacteria of a new kind in their roots. Could such bacteria be persuaded to live with one of the new high yield tropical climate grains by modifying the

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